Author’s response to reviews

Title: Prevention and reversal of selenite-induced cataracts by N-acetylcysteine amide in Wistar rats

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Reviewer #1. The article "Prevention and reversal of selenite-induced cataracts by N-acetylcysteine amide in Wistar rats" is an interesting study sufficing the need of the hour i.e use of antioxidants to slow down the development of senile cataract. However, as the limitation of the study suggest that it is an acute study, it should be taken further for more in-vivo studies. Also, the pharmokinetic profile, i.e. absorption studies of the active drug mimicking human lens should be done. The article has been very well written and not much corrections are needed.

* Page 15, Result Table 3, the units of the activity of the enzyme should be given in the tables.

We appreciate your comments. As suggested by reviewer, our future studies will focus on investigating the effectiveness of NACA eye drops in different animal models of cataract. We are investigating the pharmacokinetic profile of the drug using primary cell lines and in future would extend it to human lenses in vitro.

On page 15, Results Table 3, the units (mU/mg of protein) of the activity of the enzymes were added.

Reviewer #2. It has come to my attention that a doctoral dissertation entitled "Effects of n-acetylcysteine amide in preventing/treating cataracts" was published in 2015 by the first author, Yasaswi Maddirala. In this thesis, the biochemical levels of GSH, GSSG, MDA and GR in a 9 week experiment showed a different result at week 9 compared to that of the 30 day experiment
of this manuscript. It might be good to make some mention of the differences in the extended dissertation data and its implications.

We appreciate your careful consideration of our work. We did perform several preliminary studies using this animal model and different study designs.

For the above mentioned preliminary studies the rats were divided into four groups, (1) control, (2) NACA, (3) Na2SeO3, and (4) NACA + Na2SeO3, such that each group had one lactating female rat with 10 male pups. On postpartum day 10, all rat pups in the Na2SeO3 and NACA + Na2SeO3 groups received an intraperitoneal injection of sodium selenite (19 µmol/kg body weight), whereas the control and NACA groups received equal volumes of phosphate buffer (pH 7.4). One percent NACA eye drops, prepared in phosphate buffer (pH 7.4), were started from postpartum day 15 (the day that the rat pups opened their eyes) until week 9. Note that intraperitoneal injection of NACA was eliminated in this preliminary study. At the end of this preliminary study, biochemical parameters of oxidative stress like GSH, GSSG, GSH/GSSG, GR activity, and MDA were measured. The levels of GSH, GSSG and GSH/GSSG in the Na2SeO3 group showed no significant difference from the control group lenses. The GR activity in the Na2SeO3 group was almost similar to that of the control group, indicating the activation of lens defense systems against the oxidative stress induced by selenite. This was in accordance with previous studies that reported a similar regeneration of GSH to near control levels by day 20-25 post selenite injection [1-3]. The results from these studies were eliminated from the current manuscript to avoid confusion to the readers due to a difference in the study design. There were two major limitations of this study: 1) GSH regeneration and 2) NACA eye-drops cannot be used for prevention of cataracts in sodium selenite model as cataracts develop by day 15 when the rat pups opened their eyes.

To eliminate the influence of GSH regeneration in this model and to study the effectiveness of NACA we decreased the length of the study to 4 weeks in the current study. To study prevention of cataracts we had to include NACA i.p. injection prior to Na2SeO3 injection as well as every other day till the pups opened their eyes. Furthermore, in our future studies, we plan on using other models of cataract formation where we could focus on the effectiveness of NACA eye drops for an extended period of time.
References

